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Inhalt:

I. Wissenschaftliche Mitteilungen.

1. Lankesterella tritonis, n. sp., a Haemogregarine from the blood of the Newt, Triton cristatus (Molge cristata).

By H. B. Fantham, B. Sc. Lond., A.R.C.S., University College, London.

(With 17 figs.)

eingeg. 7. Juni 1905.

Since 1882, when Prof. Ray Lankester described the parasite of the frog's blood, *Lankesterella* (*Drepanidium*) *ranarum* [6], first seen by him some eleven years before, interest in the *Haemosporidia* has greatly increased, especially since the elucidation of the life-history of the human malaria parasite a few years ago. Researches, however, are still needed on these parasites in the cold-blooded Vertebrata.

Recently, while working at the crested newt, *Triton cristatus* (*Molge cristata*), minute bodies in the red blood corpuscles were noticed, which seemed distinctly worthy of further investigation. They appeared to be *Haemosporidia*. Accordingly, I have prepared fresh blood-films from some twenty adult newts, both male and female, and stained them in various ways, more especially by the Romanowski method, or with Delafield's haematoxylin. I have also used methylene blue in aqueous

solution with great success. Of the several modifications of the Romanowski method I have combined those of Laveran [8] and Plimmer [11], namely, using Bleu Borrel, erythrosin and tannin orange, after fixation with absolute alcohol. The use of the tannin orange is optional. This method gave good results for the detection of the parasite and its general structure, though for the nuclear (chromatic) detail of the parasite absolute alcohol is perhaps not an ideal fixative. I have found a mixture of saturated aqueous corrosive sublimate two parts, with absolute alcohol one part, as suggested by Hintze [4] after Schaudinn, to give very good results as a fixative. The preparations may then be stained with Delafield's haematoxylin, Heidenhain's iron-haematoxylin, or gentian-violet, using eosin as a successful plasma stain.

Fig. 1. Fig. 2.

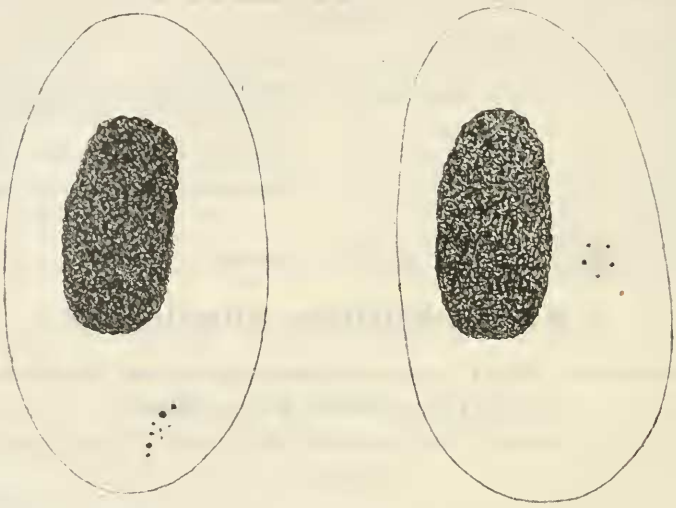
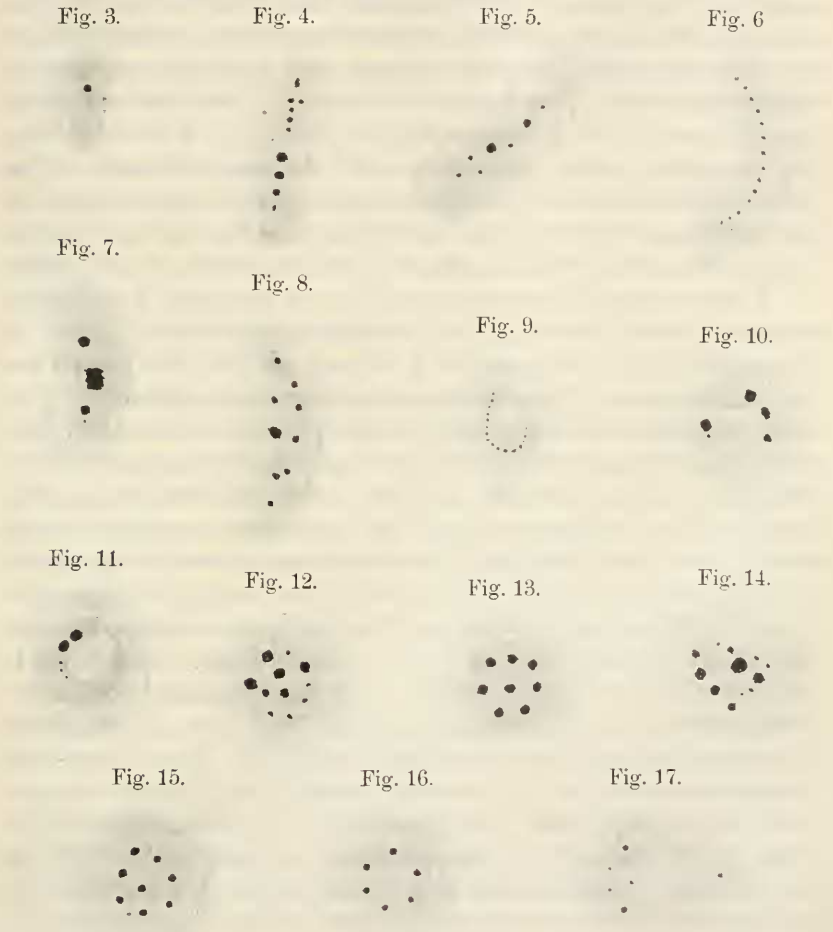


Fig. 1. Blood corpuscle of newt, with vermiform haemogregarine in situ; × 2100 approx.
Fig. 2. Same, with schizont dividing into merozoites; × 2100 approx.

I have also tried the Romanowski stain after corrosive sublimate and alcohol fixation with fair results. It may be here noted that the haematoxylins already mentioned require a long time for action in order to stain the parasite at all deeply. It is not too long to leave the films for several hours in concentrated Delafield's haematoxylin, while from sixteen to twenty four hours is necessary with a dilute acidulated solution of this stain, even longer than advocated by Hintze in the case of *L. ranarum*. About the same time is required for the action of these stains after fixation with osmic acid, or mixtures containing it.

Some time after beginning this work my attention was drawn to

the fact that Dr. A. C. Stevenson, of the Pathological Department, University College, had just begun to examine the newt's blood. He also independently found the parasite described in this paper and corroborated many of my observations. I would gratefully acknowledge his extreme courtesy in showing me his preparations. Dr. Stevenson used the Leishman stain, which I have also tried with success.



Figs. 3 to 17. Various stages in the life history of the haemogregarine, drawn under a magnification of 2100; drawings subsequently enlarged twice. In each figure the parasite is shown surrounded by some of the cytoplasm of the blood-corpuscle, as a background. The outline of this background should in all cases be soft and irregular. For further explanations, see the text.

In addition to blood-films, thin smear-preparations of the spleen-pulp and liver, and sometimes of the kidney, bone-marrow and

lung were made. The parasite was found in rather more than half the newts, in some cases 80 per cent of the red blood corpuscles being infected. Double infection of corpuscles sometimes occurred. We are here dealing with large red blood corpuscles infected with very small parasites. (figs. 1 and 2). The long diameter of the erythrocytes of the newt averages from 29 to 30 μ, while the parasite varies in size from 2 to 6 μ, as stated in greater detail below. On this account then it will be seen that the work has not been easy, and the investigation is being continued. However, the observations up to the present seem sufficiently advanced to warrant publication. I have only, with certainty, seen the trophozoites and schizogony of this haemogregarine, especially the schizont dividing into merozoites, forming "rosette stages". At present the material has yielded fewer examples of vermiform or crescentiform stages, though the observations have extended over more than three months, and on newts procured from four different agencies in the neighbourhood of London.

I have examined the parasite with Zeiss' 2 mm and 3 mm lenses, aperture 1,40 mm, and with Zeiss's compensating oculars 8, 12 and 18, more especially the combinations 2 mm lens and 12 ocular, and 3 mm lens and 18 ocular. The vermiform stages vary in length from 5 to 6 μ when apparently full-grown, and average 1 to 1,5 μ in breadth. The young trophozoites are about 2 μ in length (fig. 3). Several modes of distribution of the chromatic material in the vermiform trophozoites occur, as shewn by the stains used. Sometimes there are several deeply staining dots, often about eight in number; such forms are apparently trophozoites (figs. 4 and 5). In other cases fine closely packed granules are generally distributed throughout the cytoplasm of the parasite; such are perhaps microgametocytes (fig. 6). I have often noticed these in preparations of the spleen. Others again (but at present only a few examples have been seen) have comparatively large chromatic dots, fewer in number than in the cases previously mentioned. These are possibly macrogametocytes (fig. 7). A minute vacuole may perhaps occur in some forms of trophozoite. Some crescent-shaped forms, broader in the centre, are shown in fig. 8. Similiar figures are given by Labbé [5] in the case of his *Drepanidium princeps* of the frog but I would emphasize the difficulty of clearly defining the exact extent of the cytoplasm of this haemogregarine. In a successfully stained preparation by the Romanowski method, the cytoplasm of the parasite appears to be of a pale blue tinge. When a special plasma stain is not used after haematoxylin, the cytoplasm of the parasite is seen to be highly refractile, and even on using eosin, little, if any, of the eosin is taken up by the cytoplasm of the parasite. But it is characteristic of Sporozoa to find their protoplasm difficult to stain.

The fully developed trophozoite (schizont) bends on itself, becoming U-shaped (figs. 9 and 10), until its ends appose (fig. 11) and fuse. The nucleus of such a ring shaped schizont, at first lying chiefly at one side, begins to break up, usually into unequal portions (figs. 12 and 14), especially as seen in specimens stained by the Romanowski method. There are often eight or nine such parts or dots seen (fig. 13). Around each nuclear portion some refractile protoplasm of the parasite aggregates, and so "rosette"-stages are formed (figs. 15 and 16), consisting of merozoites in contact forming a spheroidal mass. The diameter of such a schizont, dividing into merozoites, is from 2,5 to 3,5 μ approximately. The latter, when fully formed, separate. Curious arrangements of partially separated merozoites have been seen (fig. 17).

Regarding free stages of this parasite in the blood-plasma of the host, I am of opinion that such occur in my preparations, but extreme caution needs to be exercised in making definite statements on such matters.

I have found parasites in the spleen-pulp of infected newts, and in some cases, especially well marked in one, the spleen appeared enlarged.

As to the sporogony of this haemogregarine unfortunately I have as yet no observations. In view of Siegel's recent paper on the sporogony of *Haemogregarina stepanovi* [13], and Schaudinn's remark on that of *Karyolysus lacertarum* [12] appended as a footnote to Siegel's paper, it appears highly probable that the sporogony of this haemogregarine of the newt occurs in an intermediate host, a blood-sucking organism, perhaps a leech. The idea that the sporogony of *Lankesterella ranarum* (*L. minima* of Hintze) takes place in the intestinal epithelium of the same host [4], is apparently incorrect. A parasite from the intestinal epithelium of the newt was described by Steinhaus as long ago as 1891, under the name of *Karyophagus* (*Cytophagus*) *tritonis* [14]. In Wasieliewski's "Sporozoenkunde" [15] this is placed under the *Acystosporidia*. Minchin [10, p. 270] states that this is the *Eimerian* stage of a Coccidian, and Schaudinn suggests that the parasites in the intestinal cysts of the frog mentioned by Hintze were Coccidian or some such form [12]. The intestinal tracts of many of the infected newts have been preserved and an examination of the same is in progress. In view of Schaudinn's and Siegel's researches I hardly expect to obtain a connection between the life-histories of a *Karyophagus*-like form and the haemogregarine described in this paper.

As to the systematic position of this parasite I would place it among the haemogregarines, as its trophozoite is vermiform and endoglobular in the early stages. From its size the genus *Piroplasma* is perhaps suggested, but the trophozoites are vermiform, and a rosette

stage occurs in schizogony. Following Minchin [10] in his classification of the *Haemosporidia*, this parasite would be placed in the sub-order *Haemosporea*, genus *Lankesterella*, since it is considerably less than three-fourths the length of the blood corpuscle it inhabits. As to species, it occurs in the newt, and is smaller than *L. ranarum*, so it seems distinctly worthy of a separate specific name, and I propose that of tritonis[1]. This specific name seems a more euphonious genitive than one derived from *Molge*, apart from the perplexing question as to whether the generic name of the host is *Triton* or *Molge*. In passing we might emphasize the simplicity of Laveran's classification of the *Haemosporidia* [9], when this parasite of the newt would be classed under his genus *Haemogregarina*. Mere differences of size according as to whether the parasite is three-quarters, equal to, or greater than the long diameter of the blood-corpuscle of the host, seem rather unsatisfactory as bases for generic distinctions, especially on remembering the variations in size of the corpuscles of the cold-blooded Vertebrata. The parasite herein described is apparently the smallest haemogregarine yet noted.

As far as I know, and I have been at considerable trouble to consult the literature on the subject, no account of this haemogregarine in the newt has hitherto been published. Gaule [2] in 1881 mentioned that he saw a parasite like Lankester's *Drepanidium ranarum* in a *Triton*. The species of *Triton* he leaves unmentioned, and he gives no, description, figures, or further mention of the organism, which he failed to recognize as a Sporozoon, apparently considering it a metamorphosis product of the blood-corpuscle, a so-called Cytozoon.

I am aware of the remarks of Laveran on "pseudo-haematozoa" and of Bremer on "paranuclearkörperchen", but have taken care to eliminate all slides showing imperfect fixation, or in any way doubtful. I have also read several of Jolly's admirable papers on the erythrocytes of *Triton*.

In conclusion, I would then call this parasite *Lankesterella tritonis*. It is apparently very like *L. ranarum* (*L. minima* of Hintze) but smaller, though I have not been able to procure infected frog's blood containing *L. ranarum*.

I have much pleasure in thanking Mr. A. S. Hirst for assistance in the observation of this parasite.

I would again mention that I am continuing the researches on this parasite, and intend to immediately extend the investigations to allied possible hosts.

[1] Slight variations in size occur in some cases in this parasite. However have not, on this account, followed the example of Labbé in his treatment of *L. ranarum* in making more than one species.

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2. Agaricoides, a new type of Siphonogorgid Alcyonarian.

By Jas. J. Simpson, M. A., Zoological Department, University of Aberdeen.

(With 19 figs.)

eingeg. 10. Juni 1905.

This beautiful and apparently unique Alcyonarian (Siphonogorginae) was included in a collection of Deep-sea forms obtained in the Indian Ocean by the Royal Indian Survey Ship "Investigator". As some time must elapse before the Report on the entire collection is published by the Indian Museum, it has been thought advisable to give a separate record of this peculiarly interesting type. I am indebted to Prof. J. Arthur Thomson, University of Aberdeen, and to Prof. A. Alcock, Calcutta, for the opportunity of describing it.

Fig. 1. Typical colony. (4/5 N. S.)

The new genus is represented by numerous specimens varying greatly in size which illustrate different stages of growth (figs. 1 and 4).